REMARKS

Claims 15-25 are cancelled as drawn to non-elected inventions. Applicants reserve the rights to file one or more continuation applications directed to the non-elected inventions.

Claims 1 and 29 are amended to recite that the proteorhodopsin is cellular membrane-free. Support for the amendment can be found at page 11, lines 12-13. Claims 1 and 29 are further amended to recite that the proteorhodopsin is in a monomer or an oligomer form. Support for the amendment can be found, for example, in the original Claim 12. Claim 12 is consequently amended to a dependent claim of Claim 1.

Claim 10 is amended to correct the antecedent basis.

Claim 11 is amended to recite two different proteorhodopsin variants, to clarify the meaning of the claim.

Claim 26 is amended to recite that directing light onto only a selected portion of a material containing immobilized proteorhodopsin. Claim 28 is amended to recite directing light onto only selected locations and selected layers of a three-dimensional optical information storage material. The amendments are to clarify the meaning of the claims as suggested by the Examiner.

New Claim 32 is supported by page 11, lines 12-13 and original Claim 12.

No new matter is added in any of the amendments. The Examiner is requested to enter the amendments and re-consider the application.

Telephone Interview with the Examiner

Applicants thank the Examiner for the informal telephone interview dated May 4, 2007. During the interview, the Examiner clarified his position and suggested some amendments. In order to accelerate the allowance of the application, Applicants have made further amendments to the claims.

Applicants respectfully request that in the event that the Examiner finds the amended claims still not allowable, the Examiner call the Applicants to discuss the issues such that the prosecution can be expedited.

102(a) Rejections

9. Claims 11-14, 26 and 28 are rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Dioumaev et al. "Proton Transfers in the photochemical reaction cycles of proteorhodopsin", Biochem., Vol. 41(17) pp. 5348-5358 (4/2002). The rejection is traversed because Dioumaev et al. do not teach each and every element as set forth in Claims 11-14, 26 and 28, either expressly or inherently.

Dioumaev et al. only disclose the measurements of photocycle kinetics of proteorhodopsin (PR) **using PR membranes encased in polyacrylamide gels or using membrane suspensions**.

Claim 11 is directed to a fraud-proof material comprising at least two different immobilized proteorhodopsin variants having different maximum absorption wavelengths. Dioumaev et al. do not teach the claimed element of two different proteorhodopsin variants having different spectral properties encased in polyacrylamide gels. Therefore, Claim 11 is not anticipated by Dioumaev et al.

Claim 12 is directed to an optical information carrier comprising a solid material having immobilized proteorhodopsin, wherein said proteorhodopsin is detergent-solubilized, cellular membrane-free and in a monomer or an oligomer form. Dioumaev et al. only used the membrane suspension in which PR was not cellular membrane-free and was not in a monomer or an oligomer form. Therefore, Claim 12 and its dependent Claims 13 and 14 are not anticipated by Dioumaev et al.

Regarding to Claims 26 and 28, Applicants do not agree with the rejection. However, to accelerate the allowance of the claims, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 102(a) rejection of Claims 11-14, 26 and 28 over Dioumaev et al. should be withdrawn.

10. Claims 1-4, 8-14, 26, 28 and 29 are rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Friedrich et al. "Proteorhodopsin is a light driven proton pump with variable vectorality", J. Mol. Biol., Vol 321(5) pp 821-838 (2002).

The cited claims are not anticipated by Friedrich et al. because Friedrich et al. do not teach each and every element as set forth in the cited claims.

Regarding Claims 1-4, 8-10 and 12-14, Friedrich et al. do not teach a solid material having immobilized PR, wherein the PR is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form.

"By immobilization," the PR molecules are fixed to a solid material and do not diffuse or diffuse very slowly within the solid material, such that the optical signal is not lost by diffusion of the PR molecules. (see Application at page 6, lines 26-28)

There is no dispute that Friedrich et al. generated all of the spectroscopic data in a solution phase, not in a solid phase, using PR reconstituted in phospholipid membrane vesicles. The PR molecules in Friedrich et al. are not fixed to a solid, and are not immobilized.

Therefore, Claims 1-4, 8-10 and 12-14 are not anticipated by Friedrich et al.

As to Claim 11, Friedrich et al. do not teach the claimed elements of two different proteorhodopsin variants or immobilized PR in a fraud-proof material.

As to Claims 26 and 28, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 102(a) rejection of Claims 1-4, 8-14, 26, 28 and 29 over Friedrich et al. should be withdrawn.

103(a) Rejections

11. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Friedrich et al., in view of Hampp et al. '279 and Krebs et al., "Detection of fast light activated H+ release and M intermediate formation from proteorhodopsin", BMC Physiology, Vol. 2 pp 5-12 (2002).

As discuss above, Friedrich et al. do not teach or suggest a solid material having immobilized PR, which is detergent-solubilized, cellular membrane-free PR in a monomer or oligomer form.

Hampp et al. use native purple membrane patches, which are micrometer sized patches containing a 2D crystal of lipids and bacteriorhodopsin (BR) proteins. Hampp et al. do not teach or suggest PR, let alone cellular membrane-free, detergent-solubilized

PR. Hampp et al. do not even teach or suggest cellular membrane-free, detergent-solubilized BR, because it is unstable and not suitable for optical information carrier.

The disadvantages of BR are described in the application at page 1, lines 19-27: "One of the problems with the BR-based films is that BR forms 0.2-1 μm sized protein-lipid patches. If BR is extracted from these patches to form a monomeric protein, it becomes unstable and is inactivated in a few days. The problem with using these BR patches in optical films is that the patches are approximately the same size as the wavelength of the light used to interface the film. This results in significant light scattering during read and write cycles, thereby increasing noise and degrading the performance of the film. Additionally, the BR patches tend to stick to each other, which result in uneven distribution of the BR protein in the film, and further degrade the performance of BR-based optical films."

The advantages of PR over BR are described in the application at pages 6 and 7. "One advantage of using proteorhodopsin as an optical information carrier is that proteorhodopsin can be functionally expressed in E. coli to produce a large quantity (grams or kilograms) of protein economically and efficiently." "As an optical data storage material, it is desirable to immobilize membrane-free, detergent-solubilized proteorhodopsin to avoid light scattering. Detergent-solubilized proteorhodopsin is usually in the form of a monomer, and sometimes in the form of an oligomer (dimer, trimer, tetramer, pentamer, or hexamer). Different from bacteriorhodopsin, proteorhodopsin protein is stable in its monomeric or oligomeric state for at least one month at room temperature, or one year at 4°C."

PR can be stably extracted form membrane by detergents, whereas solubilized BR is knows to have a short shelf life. There is no reasonable expectation of success for substituting BR patches in Hampp et al. with detergent-solubilized, cellular membrane-free PR, which is in a monomer or oligomer form, in an optical information carrier. **There is no reasonable expectation of success that detergent-solubilized, cellular membrane-free, monomer/oligomer form of PRs can be immobilized to a solid and have a desirable properties as an optical information carrier.**

There are many differences between the present invention and Krebs. The most important difference is that Krebs et al. describe a basic research that examines the

physical properties of PR in a solution phase; PR is not fixed to a solid and is not in an immobilized format. Krebs et al. did flash photolysis with reconstituted PR in a solution phase. Krebs et al. do not teach or suggest a solid material having immobilized PR, wherein the PR is detergent-solubilized, cellular membrane-free and in a monomer or oligomer form.

Krebs extract PR from membrane with a detergent β-octyl-D-glucoside. The column-purified PR was reconstituted into mixed micelles containing 1,2-diheptanoyl-SN-glycero-3-phosphocholine (DHPC), a phospholipid.

At page 6, last paragraph, Krebs et al. state that "The requirement for pR to be in lipid to show fast H^+ release and M formation stems either from a protein/lipid interaction needed to establish a stable, active tertiary structure, or from the need for the phosphate group in DHPC to act as a proton release group." In the second paragraph of Conclusion at page 7, Krebs et al state "The necessity of reconstituting pR with some lipid before it is capable of photocycling shows that the presence of lipids facilitates pR in assuming its fully active structure." Krebs et al state that pR needs to be reconstituted with lipids before being capable of photocycling/M state formation, thus Krebs et al, teach away from the present invention of an optical information carrier comprising immobilized PR, which is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form. On the contrary, in the present application, Applicants have provided a working example of optical data storage using proteorhodopsin-PVA film, where the PR is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form. (See application, Example 9).

In the Office Action at page 9, the Examiner states that the PR sample of Krebs et al are stable for several months. This is incorrect. The Krebs paper (at the beginning of page 3) states that PR stored for a few weeks in octylglucoside solution at 4°C has changed mobility on an SDS-PAGE gel, which indicates the instability of their PR sample during storage in the octylglucoside solution.

The detergent-solubilized, cellular membrane-free, monomer/oligomer form of PR has unexpected advantages over the membrane fragments-containing PR, or phospholipid vesicle-containing PR (reconstituted PR) in that the former does not cause

light scattering, thus providing a good signal-to-noise ratio (see Application at page 3, lines 25-29).

Therefore, Friedrich et al., Hampp et al., and Krebs et al., alone or in combination, do not teach or suggest an optical information carrier comprising a solid material comprising immobilized, detergent-solubilized, cellular membrane-free, and monomer/oligomer form of PR (Claims 1-10, 12-24, and 29-30).

As to Claim 11, Friedrich et al., Hampp et al., and Krebs et al., alone or in combination, do not teach or suggest the claimed element of two different immobilized proteorhodopsin variants in a fraud-proof material.

Applicants are not aware of any prior art describing the use of two different rhodopsin variants simultaneously. BRs from different organisms are almost identical in sequence and have the same absorbance spectra. **Unlike BR variants, PR variants have different spectral properties and different colors** (See Application at page 11, line 32 through page 12, line 1 and Table 1). Thus, prior to the present invention, there were no reasons or advantages of mixing two rhodopsin variants with different sequences. By mixing two proteorhodopsin variants with different spectral properties, it is possible to have visually different color shifts depending on the wavelength of light used to expose the solid material containing the proteorhodopsins. One proteorhodopsin variant will be selectively converted from the basal state to the M state, while the other one will predominantly remain in the basal state.

As to Claims 26 and 28, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Friedrich et al., Hampp et al. and Krebs et al. should be withdrawn.

12. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Friedrich et al., in view of Hampp et al. '279 and Krebs et al., further in view of Wu et al. "Bacteriorhodopsin encapsulated in transparent solgel glass: A new biomaterial", Chem. Mater., Vol. 5 pp. 115-120 (1993).

Wu disclose BR encapsulated in sol-gel glass. Wu et al. do not mention proteorhodopsin. As discuss above, There is no reasonable expectation of success that

detergent-solubilized, cellular membrane-free, monomer/oligomer form of PRs can be immobilized to a solid and have a desirable properties as an optical information carrier.

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Friedrich et al., Hampp et al., Krebs et al., and Wu et al. should be withdrawn.

13. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hampp et al. '279, in view of Krebs et al.

Hampp et al. do not teach or suggest PR. There is no reasonable expectation of success for substituting BR patches with detergent-solubilized, cellular membrane-free PR, which is in a monomer or oligomer form, in an optical information carrier.

Krebs et al. did flash photolysis with reconstituted PR in a solution phase. Krebs et al. do not teach or suggest a solid material having immobilized PR, wherein the PR is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form.

There is no reasonable expectation of success that detergent-solubilized, cellular membrane-free, monomer/oligomer form of PRs can be immobilized to a solid and have desirable properties as an optical information carrier, e.g., being stable and having minimal light scattering.

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Hampp et al. in view of Krebs et al. should be withdrawn.

14. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hampp et al. '279, in view of Krebs et al., further in view of Wu et al.

For the same reasons stated above in Sections 12 and 13, Claims 1-14 and 26-30 are not obvious over Hampp et al., Krebs et al., and Wu et al.

Response to Advisory Action

Applicants wish to clarify Applicants' position in response to the Examiner's statements.

The Examiner states that "When the matrix materials (PVA, gelatin, etc) is added there is every reason to expect increased stability." Applicants respectfully do not agree. Applicants' detergent-solubilized, cellular membrane-free PRs are in a macromolecular

structure such as in a trimer form covered by a detergent. When PR is immobilized onto matrix materials, if the matrix materials adsorb the detergent, the embedded PR may lose the bound detergent, which will cause the denaturization of the PR protein. There is no reasonable expectation of success of immobiling detergent-solubilized, cellular membrane-free, monomer/oligomer form of PR onto a solid until reduction to practice. Applicants have provided a working example (Example 9) of optical data storage using proteorhodopsin-PVA film, where the PR is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form.

The Examiner states that "The applicant's position that octyl-beta-D-glucoside is not a detergent and that it cannot solubilize pR monomers." This is not what Applicants have stated. Applicants actually stated in the previous Response: "DHPC is a phospholipid that can form micelles. DHPC is not a detergent that can solubilize pR from membrane." DHPC is the abbreviation of 1,2-diheptanoyl-SN-glycero-3-phosphocholine.

The Examiner states that the detergent-absorbing beads absorb excess of dioleophospholipid, which is a detergent. Applicants restfully do not agree. The beads absorb the detergent used for solubilizing the membrane, probably either octyl-beta-D-glucoside (OG) or dodecyl maltoside (DM). In the experiment, the OG/DM solubilized PR was simultaneously mixed with dioleoly-phospholipids and detergent-absorbing beads. The beads bind OG/DM, and PR was simultaneously incorporated into the dioleoly-phospholipid vesicles. This is because PR is very apolar, and therefore, in the absence of OG/DM, it prefers to become incorporated into lipid vesicles. It is unlikely that the detergent-absorbing beads will bind a significant amount of dioleoly-phospholipids.

CONCLUSION

Applicant believes that the application is in good and proper condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 798-3570.

Respectfully submitted,

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